PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
A61K 31/155, 39/395, A61P 37/02 // (A61K 39/395, 31:155)

A1

(11) International Publication Number: WO 00/61132

(43) International Publication Date: 19 October 2000 (19.10.00)

(21) International Application Number: PCT/US00/10253

(22) International Filing Date: 14 April 2000 (14.04.00)

(30) Priority Data:
09/291,712 14 April 1999 (14.04.99) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 09/291,712 (CIP)
Filed on 14 April 1999 (14.04.99)

(71) Applicants *(for all designated States except US)*: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, OF AMERICA DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health Office of Technology Transfer, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US). THE UAB RESEARCH FOUNDATION [US/US]; 1120-G Administrative Building, 701 South 20th Street, Suite 1120-G, Birmingham, AL 35294-0111 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: NEVILLE, David, M. [US/US]; 9624 Parkwood Drive, Bethesda, MD 20814 (US). THOMAS, Judith, M. [US/US]; 2117 Brook Highland Ridge, Birmingham, AL 35242 (US).

(74) Agents: SPRATT, Gwendolyn, D. et al.; Needle & Rosenberg, P.C., Suite 1200, 127 Peachtree Street, N.E., Atlanta, GA 30303-1811 (US).

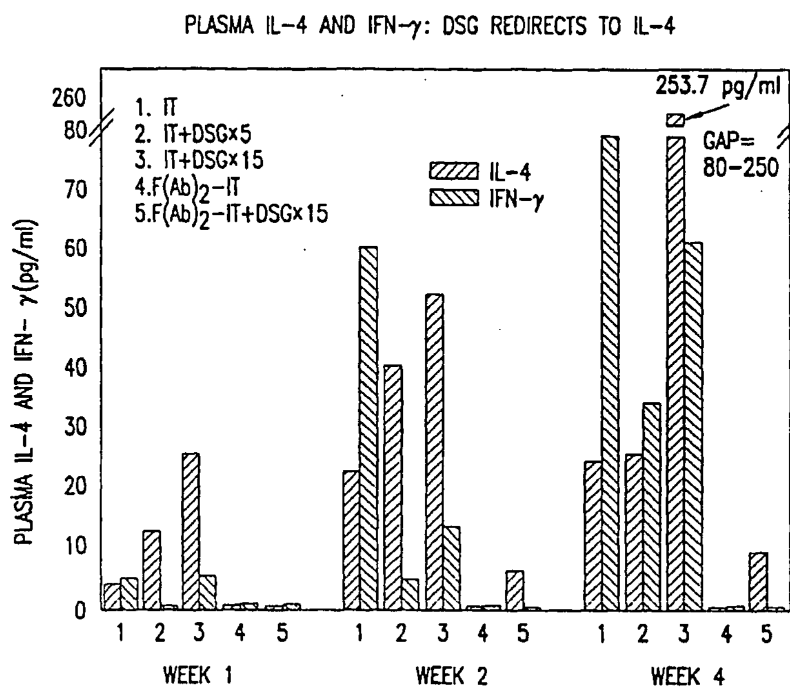
(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: COMPOSITIONS CONTAINING IMMUNOTOXINS AND AGENTS THAT INHIBIT DENDRITIC CELL MATURATION FOR INDUCING IMMUNE TOLERANCE TO A GRAFT

(57) Abstract

The present invention provides a method of inducing immune tolerance to a graft in a recipient, comprising administering to the recipient an immunotoxin, thereby reducing the recipient's T-cell population; and administering to the recipient an agent that inhibits dendritic cell maturation. The present invention also provides a method of screening for an agent that acts synergistically with an immunotoxin in inducing immune tolerance and a method of screening for an agent that inhibits dendritic cell maturation. The present invention also provides a method of treating a subject with an autoimmune disease, comprising administering to the subject an immunotoxin, thereby reducing the subject's T-cell population; and administering to the subject an agent that inhibits dendritic cell maturation. Also provided herein is a composition comprising an immunotoxin and an agent that inhibits dendritic cell maturation.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

COMPOSITIONS CONTAINING IMMUNOTOXINS AND AGENTS THAT INHIBIT DENDRITIC CELL MATURATION FOR INDUCING IMMUNE TOLERANCE TO A GRAFT

BACKGROUND OF THE INVENTION

Field of The Invention

This invention relates to techniques for inducing immune tolerance using an immunotoxin combined with an agent that inhibits dendritic cell maturation. The invention is in the field of immunobiology.

Background Art

Transplant tolerance remains an elusive goal for patients and physicians whose ideal would be to see a successful, xenogeneic transplantation performed without the need for indefinite, non-specific maintenance immunosuppressive drugs and their attendant side effects. As with many transplant procedures, securing viable allogeneic grafts can be difficult. In addition, long term immunosuppression can be problematic.

Over the past 10 years, the majority of transplant recipients have been treated with cyclosporin, azathioprine, prednisone, and a variety of other immunosuppressive agents for maintenance immunosuppression. The average annual cost of maintenance immunosuppressive therapy in the United States is approximately $10,000. In addition to the cost, these agents, because of their non-specific effects, have considerable side effects, including compromising cell and organ function and increasing susceptibility to infection. A major goal in transplant immunobiology is the development of specific immunologic tolerance to transplants with the potential of freeing patients from the side effects of continuous pharmacologic immunosuppression and its attendant complications and costs.

Anti-T cell therapy (anti-lymphocyte globulin) has been used in rodents in conjunction with thymic injection of donor cells (Posselt et al. *Science* 1990; 249: 1293- 1295 and Remuzzi et al. *Lancet* 1991; 337: 750-752). Thymic tolerance, which

has proved successful in rodent models, involves the exposure of the recipient thymus gland to donor alloantigen prior to an organ allograft from the same donor. However, thymic tolerance has never been reported in large animals, and its relevance to tolerance in humans in unknown.

One approach to achieving immune tolerance has been to expose the recipient to cells from the donor prior to the transplant, with the hope of inducing tolerance to a later transplant. This approach has involved placement of donor cells (e.g., bone marrow) presenting MHC Class I antigens in the recipient's thymus shortly after application of anti-lymphocyte serum (ALS) or radiation. However, this approach has proved difficult to adapt to live primates (e.g., monkeys or humans). ALS and/or radiation render the host susceptible to disease or side-effects and/or are insufficiently effective.

If a reliable, safe approach to specific immunologic tolerance to transplantation, particularly xenogeneic transplantation, could be induced, this would be of tremendous value and appeal to patients and transplant physicians throughout the world, with immediate application to new transplants and with potential application to existing transplants in recipients with stable transplant function. Thus, a highly specific immune tolerance inducement is desired. Furthermore, there is a need for a means for imparting tolerance in primates, without the adverse effects of using ALS or radiation. Moreover, the goal is to achieve tolerance rather than simply delaying the rejection response. An important goal is to inhibit the rejection response to the point that rejection is not a factor in reducing average life span among transplant recipients.

SUMMARY OF THE INVENTION

The present invention provides a method of inducing immune tolerance to a graft in a recipient, comprising administering to the recipient an immunotoxin, thereby reducing the recipient's T-cell population; and administering to the recipient an agent that inhibits dendritic cell maturation. The agent that inhibits dendritic cell maturation

is administered to the recipient at least once and, preferably, on the day of transplantation. Even more preferably, the agent that inhibits dendritic cell maturation is administered to the recipient an additional four to fourteen times over the course of one to two weeks following transplantation. Optionally, the agent that inhibits dendritic cell maturation can be administered to the recipient prior to transplantation, and/or to the donor prior to harvesting the graft. The agent that inhibits dendritic cell maturation can be an inhibitor of nuclear translocation of NfκB, including, for example, deoxyspergualin, methyl-deoxyspergualin, and other deoxyspergualin derivatives or analogs. Other agents that inhibit dendritic cell maturation can include, for example, a soluble interleukin 17 (IL-17) receptor Fc fusion protein, a glucocorticoid, a blocker of tumor necrosis factor alpha binding, a blocker of granulocyte macrophage colony stimulating factor binding, a blocker of IL-12p70 binding, or a blocker of IL-1β binding. An agent that inhibits dendritic cell maturation can also include a blocker of an immature dendritic cell epitope or a blocker of a dendritic cell precursor epitope involved in dendritic cell maturation such as an anti-CD40 ligand (i.e., anti-CD154), and, more specifically, the anti-CD40 ligand can be 5C8 or a derivative of analog thereof.

The present invention also provides a method of screening for an agent that acts synergistically with an immunotoxin in inducing immune tolerance, comprising transplanting a donor graft to a recipient; administering to the recipient an immunotoxin, thereby reducing the recipient's T-cell population; administering to the recipient the agent to be screened; obtaining a dendritic cell-containing sample; and determining a percentage of the dendritic cells in the sample that express or can be induced to express a marker specific for mature dendritic cells, wherein a low percentage shows a synergistic action. Also provided by the present invention is a method of screening for an agent that inhibits dendritic cell maturation, comprising obtaining a population of immature dendritic cells from a dendritic cell-containing sample of a subject; culturing the population of cells in the presence of the agent to be screened; and determining a percentage of dendritic cells that express or can be induced to express a marker specific for mature dendritic cells, wherein a low

percentage shows inhibition of dendritic cell maturation. The present invention also provides a method of treating a subject with an autoimmune disease, comprising administering to the subject an immunotoxin, thereby reducing the subject's T-cell population; and administering to the subject an agent that inhibits dendritic cell maturation.

Also provided herein is a composition comprising an immunotoxin and an agent that inhibits dendritic cell maturation. More specifically, the present invention provides a composition, wherein the immunotoxin is an anti-T cell immunotoxin directed at the CD3 epitope or wherein the agent that inhibits dendritic cell maturation is an inhibitor of nuclear translocation of NfκB.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows that kidney transplant tolerance induction by treatment of the transplant recipient with immunotoxin on days 0 and +1 in combination with DSG on days 0-14 results in a prominent Th2 cytokine polarization. By the second week post transplantation, IL4 polarization (a lower level of IL4 compared to the group treated with immunotoxin alone and lower levels of IL4 compared to IFN-γ levels) is shown in the groups that received the combined treatment. The group that received DSG for two weeks showed a sustained, progressive increase in IL4 polarization at 4 weeks, whereas, in the group that received DSG for only 5 days, the polarization was no longer sustained and the cytokine pattern was reversed. The IL4 polarization occurred independently of the type of anti-CD3 immunotoxin, i.e., the whole IgG or $F(Ab)_2$ form.

Figures 2A, 2B, and 2C show the results of RT-PCR assays performed on freshly obtained rhesus monkey peripheral blood lymphocytes treated with either whole IgG immunotoxin (FN18-CRM9), $F(Ab)_2$ immunotoxin (FN18-$F(Ab)_2$-CRM9), or control buffered saline (PBS). Figure 2A shows mRNA for IL-2, IL-4, IL-10, IFN-γ in the absence of DSG. Figure 2B shows mRNA for IL-2, IL-4, IL-10, IFN-γ in cells

treated with DSG (2.5 μg/ml). Figure 2C shows the density ratio of the various mRNAs to the actin control.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a short course, immune tolerance inducing treatment regimen utilizing an immunotoxin that, when combined with agents that inhibit dendritic cell maturation, prevents transplant rejection while maintaining transplant function. Thus, the present invention provides a method of inducing immune tolerance to a graft in a recipient, comprising administering to the recipient an immunotoxin, thereby reducing the recipient's T-cell population; and administering to the recipient an agent that inhibits dendritic cell maturation.

As used throughout, a "graft" can include an allogenic or xenogenic organ, tissue, or cellular transplant. The graft, for example, can be selected from the group consisting of kidney, liver, heart, pancreas, lung, skin, and isolated cell transplants of pancreatic islets, hepatocytes, stem cell precursors, and differentiated stem cell precursors.

As used throughout, the "recipient" or "subject" is preferably a mammal. More preferably, the mammalian recipient is a primate, and, even more preferably, a human. The "recipient" or "subject" being treated can include individual humans, domesticated animals, livestock (e.g., cattle, horses, pigs, etc.), and pets (e.g., cats and dogs).

As used throughout, a "donor" can be a cadaver or a living donor. Furthermore, the donor can be of the same species as the subject being treated or a different species than the subject being treated. Thus, using the method of the invention, transplantation can be performed across primate species (i.e., xenogeneic transplantation or xenograft) and within the same primate line (i.e., allogeneic transplantation or allograft). Even

highly sensitive xenografts can maintain function in the presence of this immune tolerance inducing regimen.

The donor grafts used in the present methods can comprise cells that are altered, such as by genetically engineering the donor or donor cells. For example, the donor cells could be engineered to reduce antigenicity or to reduce the susceptibility of transplanted cells to immune injury (R. Weiss, Nature 391: 327-28 (1998)).

As used herein, "an agent that inhibits dendritic cell maturation" refers to any agent that, when in contact with dendritic cell precursors, inhibits all or a portion of the precursors from expressing markers of mature dendritic cells. Thus, an agent that inhibits dendritic cell maturation would cause about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100% fewer cells to express markers such as membrane CD83, DR, or CD86 or nuclear Rel-B than in the absence of the agent. The effectiveness of the inhibitor of dendritic cell maturation, therefore, can be assessed by determining the percent decrease in the number of cells expressing markers for mature dendritic cells, as described more fully in the examples provided herein.

The agent that inhibits dendritic cell maturation can be an inhibitor of nuclear translocation of NfκB. More specifically, the inhibitor of nuclear translocation of NfκB can be deoxyspergualin or a derivative or analog thereof, including, for example, methyl-deoxyspergualin or a deoxyspergualin analog lacking a chiral center (e.g., LF 08-0299) (Andoins et al., 1996, which is incorporated herein by reference). Other derivatives or analogs of deoxyspergualin can be used that include, for example, those identified in U.S. Pat. No. 4,518,532; U.S. Pat. No. 4,518,532; U.S. Pat. No. 4,525,299; U.S. Pat. No. 4,956,504; U.S. Pat. No. 5,162,581; U.S. Pat. No. 5, 476,870; U.S. Pat. No. 5,637,613; W.O. 96/24579; EP 600762; EP 669316; EP 7433000; EP 765866; and EP 755380, which are incorporated herein by reference.

Preferably, the agent that inhibits dendritic cell maturation activates one or more NF-AT dependent Th2 cytokines (e.g., one or more cytokine selected from the group

consisting of IL2, IL4, and IL10). Also, the agent that inhibits dendritic cell maturation preferably inhibits one or more NfκB dependent Th1 cytokines (e.g., INFγ). Such activation or inhibition can be due to an increase or decrease, respectively, in the cytokine gene expression. Thus, alterations in levels of the mRNA encoding the cytokine or protein levels can occur with treatment with the agent. In one embodiment of the invention, the agent activates one or more NF-AT dependent Th2 cytokines and inhibits one or more NfκB dependent Th1 cytokines. Thus, for example, the agent can activate IL4 and inhibit INFγ.

Alternatively, the agent that inhibits dendritic cell maturation can be a soluble IL-17 receptor Fc fusion protein, a glucocorticoid, a blocker of tumor necrosis factor alpha (TNF-α) binding, a blocker of granulocyte macrophage colony stimulating factor (GM-CSF) binding, a blocker of IL-12p70 binding, or a blocker of IL-1β binding. An agent that inhibits dendritic cell maturation can also include a blocker of an immature dendritic cell epitope or a blocker of a dendritic cell precursor epitope involved in dendritic cell maturation such as an anti-CD40 ligand (i.e., anti-CD154), and, more specifically, the anti-CD40 ligand can be 5C8 or a derivative of analog thereof. One skilled in the art would recognize that agents that inhibit dendritic cell maturation can be used in combination for either a synergistic or additive effect.

Preferably, the agent that inhibits dendritic cell maturation is administered to the recipient at least once. Even more preferably, the agent that inhibits dendritic cell maturation can be administered to the recipient at least on the day of transplantation and 1, 2, 3, 4, 5, 6, or 7 additional days within the first week following transplantation or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 additional days within the first two weeks following the day of transplantation. The course of administration can optionally be optimized to produce a sustained increase in the activation of NF-AT transcription pathway mediated cytokines and/or the inhibition of NfκB transcription pathway mediated cytokines. Thus, in one embodiment, the agent is administered daily, on the day of transplantation and 13 or 14 additional times.

In addition to administration on and after the day of transplantation, the agent that inhibits dendritic cell maturation can additionally be administered to the recipient prior to transplantation. For example, deoxyspergualin can be administered to the recipient at least once between 24 hours and 0.25 hours, and preferably, 23, 22, 21, 20, 19, 18, 17, 16, 15,14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.5 hours, prior to transplantation. Optionally, the present invention can further comprise administering to a transplant donor, prior to harvesting the transplant, an agent that inhibits dendritic cell maturation. For example, deoxyspergualin can be administered to the donor at least once between 24 hours and 0.25 hours, and preferably, 23, 22, 21, 20, 19, 18, 17, 16, 15,14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.5 hours, prior to harvesting the graft. Although the time course for administration of inhibitor of dendritic cell maturation prior to transplantation may be limited by the unpredictability of organ availability and procurement, it is preferred to administer the inhibitor to the recipient prior to transplantation and/or to the donor prior to harvesting the graft.

The preferred dose of inhibitors of dendritic cell maturation will also vary depending on the specific inhibitor used; the species, age, weight and general condition of the recipient; the mode of administration; and the like. Thus, it is not possible or necessary to specify an exact dose, as an appropriate time course may be determined without undue experimentation by one of ordinary skill in the art. By way of example, the dose of deoxyspergualin can be between 0.1 and 10 mg/kg/d, including 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0 mg/kg/d or any amount in between.

As used throughout, an "immunotoxin" can be an anti-T cell immunotoxin directed at the CD3 epitope. The immunotoxin can be an immunoconjugate or fusion protein. The immunotoxin can be either monovalent or divalent. The divalent anti-T cell immunotoxin can be UCHT1-CRM9 or a derivative or analog thereof. The divalent anti-T cell immunotoxin can comprise a toxin moiety and a targeting moiety directed to the T cell CD3ϵ epitope, and the toxin moiety can be a diphtheria toxin. The divalent anti-T cell immunotoxin can be an engineered divalent fusion

immunotoxin. Various immunotoxins and treatment regimens are described more fully in U.S. Serial No. 09/064,413 and PCT/US 98/04303, which are incorporated herein by reference. The immunotoxin can be, for example, a whole IgG immunotoxin (such as FN18-CRM9) or can be a $F(Ab)_2$ form of the immunotoxin (such as FN18-$F(Ab)_2$ CRM9).

In the present invention, the immunotoxin is administered at least two times, preferably at least on the day of transplantation and on the second day following transplantation. The immunotoxin can be administered beginning at 72 to 0 hours before transplantation and continuing up to several days thereafter. Preferably, the immunotoxin can be administered to the recipient, to the donor, or both 72-48 hours prior to a xenogeneic transplant and 24 to 0 hours prior to an allogeneic transplant. Preferably, the immunotoxin is administered to the recipient for 1, 2, or 3 days following transplantation or any time in between. It is contemplated that immunotoxin administration of up to 4, 5, 6, or 7 days can be used, but such an extended time course of immunotoxin administration would require that the production of antitoxin antibodies, which begins after approximately 5 days of administration, be addressed. The immunotoxin can be administered in subjects beginning anytime after transplantation. Thus, it is contemplated that a subject with a long term surviving transplant, who has not previously received immunotoxin treatment, could still benefit from immunotoxin administration, thereby avoiding the need for long-term treatment with immunosuppressives. It is further contemplated that a graft recipient who begins to show signs of rejection may benefit from immunotoxin administration to reduce or eliminate the rejection process. Thus, even in recipients that have previously been treated with immunotoxins, recipients showing a rejection responses can be further treated with additional immunotoxin administration.

As used throughout, the immunotoxin preferably transiently reduces the subject's or recipient's T cells in the blood and lymph nodes by at least one log unit. Preferably the number of T cells in the blood and lymph nodes will be transiently decreased by 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, or 3 log units, or

any interval amount between 0.7 and 3 log units. By "transiently reduces" is meant that T cells are reduced by 0.7 to 3 log units in the blood and lymph compartments for at least four days before starting to return to normal levels.

The present method can further comprise administering an immunosuppressive agent to the recipient. As used throughout, an immunosuppressive agent, or immunosuppressant, includes, for example, methylprednisolone, Neoral, cyclosporine, mycophenolate moefitil, tacrolimus, azathioprine, rapamycin, a steroid, or any combination thereof. The immunosuppressive agents can be administered beginning 24 to 0 hours prior to transplantation and continuing up to two weeks thereafter. Preferably, the immunosuppressive agents can be administered for at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days, or any interval time. The regimen of combined immunotoxin and dendritic cell maturation inhibition agent allows short-term immunosuppressive therapy and eliminates the need for long-term treatment with immunosuppressives, thereby avoiding the side effects associated with chronic immunosuppression. Thus, when a course of immunosuppressants is used in conjunction with immunotoxins and an agent that inhibits dendritic cell maturation, the course of immunosuppression can be shorter in duration and/or lower in dosage than is traditionally used to prevent graft rejection.

Adjunct therapies can be used together in the present methods of inducing immune tolerance and treating immune disorders . Thus, the invention includes at least one method of inducing tolerance using immunotoxin (IT): (1) tolerance induction by administering IT in combination with an agent or agents that inhibit dendritic cell maturation; (2) tolerance induction by administering IT, an agent or agents that inhibit dendritic cell maturation, and at least one or a combination of immunosuppressant drug. The adjunct therapy can be administered before, at the same time or after the administration of immunotoxin. Different adjunct therapies can be administered to the recipient at different times or at the same time in relation to the transplant event or the administration of immunotoxin, as further described below.

Because the immunosuppressant can be administered before treatment with the immunotoxin and agent that inhibits dendritic cell maturation, the present method of combining immunotoxins and agents that inhibit dendritic cell maturation can be used with a graft recipient who is on an immunosuppressant regimen. This presents a significant opportunity to reduce or eliminate traditional immunosuppressant therapy and its well documented negative side-effects. Also, treatment with agents that inhibit dendritic cell maturation and/or immunosuppressants prior to transplantation could be particularly useful in cadaveric and xenogeneic transplants. In such a setting of pre-transplant treatment, the administration of immunotoxin can be delayed for up to seven or more days post-transplantation.

Examples of schedules of administration of an immunotoxin and an agent that inhibits dendritic cell maturation, for patients receiving organ transplants are as follows:

day -24 to -0 hours:	treat recipient with the agent that inhibits dendritic cell maturation (optional)
day 0 :	perform transplant; immediately following transplant, administer lst immunotoxin dose; treat the recipient with the agent that inhibits dendritic cell maturation
day 1 :	treat the recipient with the agent that inhibits dendritic cell maturation
day 2 :	2nd immunotoxin dose; treat the recipient with the agent that inhibits dendritic cell maturation
day 3 :	treat recipient with the agent that inhibits dendritic cell maturation
day 6 :	treat recipient with the agent that inhibits dendritic cell maturation;

or

day -24 to -0 hours:	treat recipient with the agent that inhibits dendritic cell maturation (optional)

day 0	:	perform transplant; immediately following transplant, administer lst immunotoxin dose; treat the recipient with the agent that inhibits dendritic cell maturation
day 2	:	2nd immunotoxin dose
day 4	:	treat recipient with the agent that inhibits dendritic cell maturation
day 7	:	treat recipient with the agent that inhibits dendritic cell maturation;
day 10	:	treat recipient with the agent that inhibits dendritic cell maturation;
day 13	:	treat recipient with the agent that inhibits dendritic cell maturation.

In either example, the donor can be treated with the agent that inhibits dendritic cell maturation 24 to 0 hours prior to harvesting the transplant and/or the recipients can be treated with immunosuppressants prior to transplantation, on the day of transplantation, or following transplantation. One skilled in the art would know to modify the time course according to the individual recipient, depending on the species, age, weight and general condition of the recipient, the particular agents used, the mode of administration, and the like. Thus, it is not possible or necessary to specify an exact time course. However, an appropriate time course may be determined by one of ordinary skill in the art.

The presently preferred doses of the immunotoxin are those sufficient to deplete peripheral blood T-cell levels to 80%, preferably 90% (or especially preferably 95% or higher) of preinjection levels. This should require mg/kg levels for humans similar to those for monkeys (e.g., 0.05 mg/kg to 0.3 mg/kg body weight), which toxicity studies indicate should be well tolerated by humans. Thus, the immunotoxin can be administered to safely reduce the recipients T cell population.

The effectiveness of the method of inducing tolerance can be assessed using methods well known in the art. Clinical signs of graft function and graft rejection could be assessed. For example, using an method of inducing tolerance to a pancreatic or islet cell graft, blood glucose levels could be measured, and non-fasting blood glucose levels will preferably be maintained below 160 mg/dl upon completion of the immune tolerance induction regimen would indicate the absence of graft rejection. Other signs of graft rejection include histological characteristics (for example, upon biopsy of the transplant) such as interstitial fibrosis, interstitial hyperplasia, arteriolar narrowing, and ischemic injury to the transplanted organ. Additionally, histological signs of rejection of a kidney transplant include reduplication and thickening of the glomerular basement membrane. The clinical signs of rejection include gradually progressive graft dysfunction. For example, in a subject with a kidney transplant, increasing blood levels of creatinine would indicate rejection. Normal serum levels of creatinine are less than 2.0 mg/dl, and more typically about 0.5 to 1.5 mg/dl. Renal graft dysfunction is evidenced by approximately a two fold increase in baseline levels of creatinine. The presence of antidonor antibody and increasing levels of antidonor antibody could also indicate graft dysfunction. One skilled in the art would recognize other histological indicators and clinical indicators of rejection.

The present invention also provides a method of screening for an agent that acts synergistically with an immunotoxin in inducing immune tolerance, comprising transplanting a donor graft to a recipient; administering to the recipient an immunotoxin, thereby reducing the recipient's T-cell population; administering to the recipient the agent to be screened; obtaining a dendritic cell-containing sample; and determining a percentage of the dendritic cells in the sample that express or can be induced to express a marker specific for mature dendritic cells, wherein a low percentage shows a synergistic action. As used throughout, a "dendritic cell-containing sample" can include, for example, a lymph node biopsy, a blood sample, or a tonsil biopsy. Also, as used throughout, a "marker specific for mature dendritic cells" can include, for example, membrane CD83, membrane DR, membrane CD86, cytoplasmic

P55, or nuclear Rel-B. These can be routinely detected using methods known in the art. (O'Doherty et al., 1997).

Also provided by the present invention is a method of screening for an agent that inhibits dendritic cell maturation, comprising obtaining a population of immature dendritic cells from a dendritic cell-containing sample of a subject; culturing the population of cells in the presence of the agent to be screened; and determining a percentage of dendritic cells that express or can be induced to express a marker specific for mature dendritic cells, wherein a low percentage shows inhibition of dendritic cell maturation. Preferably, the population of cells is cultured in monocyte-conditioned medium supplemented with TNFα.

The present invention also provides a method of treating a subject with an autoimmune disease, comprising administering to the subject an immunotoxin, thereby reducing the subject's T-cell population; and administering to the subject an agent that inhibits dendritic cell maturation. Autoimmune diseases that can be treated by the present method include, for example, systemic lupus erythematosus, myasthenia gravis, stiff-man syndrome, an autoimmune thyroid disease, Sydenham chorea, rheumatoid arthritis. One skilled in the art would recognize a variety of ways to assess the effectiveness of the treatment, for example, a reduction in clinical symptoms (such as decreased pain, improved muscle function, improved thyroid function) and histological or biochemical evidence of reduced T cells and or mature dendritic cells.

In the present method of treating autoimmune diseases, the agent that inhibits dendritic cell maturation is administered to the subject at least once, or two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen times, preferably over a period of about two weeks, and the immunotoxin is preferably administered at least one, two, or three times, preferably within a period of less than five days. As discussed above in the context of inducing immune tolerance, immunotoxin can be administered for about 4 days if the production of antitoxin antibodies is addressed. Alternatively, the method of treatment can further comprise administering an

immunosuppressive agent to the subject. Treatment of immunosuppressants, however, could be of limited duration and a lower dose than traditionally used.

Because the immune functions that govern graft acceptance and rejection and autoimmune diseases are similar across primate species, the combined use of immunotoxins and agents that inhibit the maturation of dendritic cells as described herein is expected to succeed in humans for inducing immune tolerance or for treating autoimmune disorders.

The present invention also provides a composition comprising an immunotoxin and an agent that inhibits dendritic cell maturation. More specifically, the present invention provides a composition, wherein the immunotoxin is an anti-T cell immunotoxin directed at the CD3 epitope or wherein the agent that inhibits dendritic cell maturation is an inhibitor of nuclear translocation of NfκB. Preferably, the agent that inhibits dendritic cell maturation activates one or more NF-AT dependent Th2 cytokines. Also, the agent that inhibits dendritic cell maturation preferably inhibits one or more NfκB dependent Th1 cytokines. In one embodiment of the invention, the agent activates one or more NF-AT dependent Th2 cytokines and inhibits one or more NfκB dependent Th1 cytokines. Thus, for example, the agent can activate IL4 and inhibit INFγ.

The composition of the present invention can further comprise a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material can be administered to a subject along with the immunotoxin and agent that inhibits dendritic cell maturation without causing any substantial undesirable biological effects or interacting in a deleterious manner with any of the components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject. Suitable carriers can include, for example, water, pyrogen free saline, pharmaceutically accepted oils, or a mixture of any of these. The carrier can

also contain other suitable pharmaceutical additions such as buffers, preservatives, flavoring agents, viscosity or osmo-regulators, stabilizers or suspending agents.

The present invention is more particularly described in the following examples which are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art.

EXAMPLE 1

Transient arrest of dendritic cell maturation for primate tolerance induction

In nonhuman primates, following T cell depletion in lymph nodes and blood after day-of-transplant induction with anti-CD3 immunotoxin, stable tolerance to MHC mismatched renal allografts is favored by inhibition of proinflammatory cytokine responses using brief treatment with the NFκB inhibitor, 15-deoxyspergualin. The mechanism for this synergistic effect is blockade of dendritic cell precursor maturation during the T cell recovery phase after immunotoxin induction.

Normal 3 kg male rhesus monkeys received a renal allograft from an unrelated, MHC mismatched donor. The monkeys were then treated with two injections of immunotoxin (100μg/kg/d) on day 0 and +2, methylprednisolone (MP) tapering from 7 mg/kg/d to zero on days 0 to +3, and deoxyspergualin (2.5 mg/kg/d) on days 0 to +4 (n=5) or 0 to +14 (n=5). Additional recipients had immunotoxin with MP alone (n=3) or MP plus Neoral (100 mg/gk/d) on days 0-4 (n=3). Inguinal and axial lymph nodes were biopsied at 5, 15, and 30 days and examined by immunohistochemistry for the presence of markers of mature dendritic cells, specifically, for membrane CD83, DR, and CD86 and nuclear Rel-B.

Renal allografts survived without histologic or clinical evidence of acute or chronic rejection in 80% of IT-treated recipients given MP plus deoxyspergualin x 15d, in 40% in those given MP plus deoxyspergualin x 5d, and in 0% of those given only MP or MP plus Neoral. The 3 longest deoxyspergualin survivors are >830 days (2.3 years) with normal renal function and immunologic evidence of specific tolerance.

Compared to lymph nodes from transplants without deoxyspergualin, the day +5 lymph node tissue from deoxyspergualin-treated recipients showed extreme reduction in mature DC, i.e., a mean 58-fold ± 9.9 reduction in membrane expression for DC83, 49-fold ± 9.0 for CD86, and 81-fold± 13.5 for DR. Rel-B nuclear positive cells were reduced 46 fold ±7.1. Partial recovery occurred by day 15, and by day 30, DR+, CD83+, and Rel-B+ cells were within control range. CD86+ cells were still reduced (by 7.5-fold±1.8). The results indicate a dose-dependent NFκB inhibition and DCp maturation arrest by deoxyspergualin.

The data show the NFκB inhibitor, deoxyspergualin, blocks maturation of dendritic cell precursors *in vivo* in nonhuman primates. We hypothesize that the unusual synergy of immunotoxin and deoxyspergualin in promoting tolerance in this difficult model is due in part to depletion of memory as well as naive T cells by immunotoxin. Conceptually, this would create a transitional circumstance in which repopulating naive T cells are committed to dendritic cell costimulatory requirements for activation, while dendritic cell precursor acquisition of costimulatory function is arrested by deoxyspergualin. The net result, a window of 2-3 weeks, is a fleeting lapse into a "non-dangerous" milieu akin to the neonate's, coincidentally reduced both in T cell scope and dendritic cell precursor maturity, thereby favoring tolerance.

EXAMPLE 2

To directly examine dendritic cell precursor maturation, isolated rhesus peripheral blood dendritic cell precursors in medium containing monocyte conditioned medium (MCM) supplemented with TNFα were examined. Deoxyspergualin (0.6-10 mg/ml) was added to dendritic cell precursor cultures prior to adding MCM/TNFα. Expression of dendritic cell maturation markers, as in Example 1, was assessed by flow cytometry and by immunocytochemistry staining of cytocentrifuge preparations.

The *in vitro* data showed a shift from nuclear to cytoplasmic and from membrane to cytoplasmic staining of Rel-B and CD83, respectively, reflecting a

dominant immature dendritic cell precursor phenotype in the deoxyspergualin treated cultures. The *in vitro* results are consistent with the *in vivo* results of Example 1.

Example 3

Allografts were performed as in Example 1. The graft recipients were treated with either the whole IgG form of the immunotoxin or the $F(Ab)_2$ form of the immunotoxin on days 0 and +1, also as described in Example 1. Some of the recipients also received DSG daily for either five days following transplantation or for two weeks following transplantation. Plasma levels of the Th2 cytokine IL4 and the Th1 cytokine interferon gamma (IFN-γ) were assayed 1 week, 2 weeks, or 4 weeks after transplantation. Following treatment with the whole IgG immunotoxin alone, levels of IFN-γ and IL-4 increased progressively from 1 to 4 weeks with levels of IFN-γ higher than levels of IL-4. When treatment with the whole IgG immunotoxin is combined with DSG treatment, the levels of cytokines change. By the second week post transplantation, a clear IL4 polarization is shown in the groups that received the combined treatment. Specifically the groups that received combined treatments showed a substantially lower level of IL4 compared to the group treated with immunotoxin alone and substantially lower levels of IL4 compared to IFN-γ levels. The group that received DSG for two weeks showed a sustained, progressive increase in IL4 polarization at 4 weeks, whereas, in the group that received DSG for only 5 days, the polarization was no longer sustained and the cytokine pattern was reversed. These data indicated that a two-week course of DSG, in combination with immunotoxin, was necessary to achieve the effect of sustained IL4 polarization. The IL4 polarization occurred independently of the type of anti-CD3 immunotoxin, i.e., the whole IgG or $F(Ab)_2$ form. The magnitude of the IL4 response was lower with the $F(Ab)_2$ form, but the polarization was virtually complete inasmuch as there was no measurable interferon-gamma. See Figure 1.

Example 4

RT PCR techniques well known in the art were used with freshly obtained rhesus monkey peripheral blood lymphocytes stimulated with whole IgG immunotoxin

(FN18-CRM9), $F(Ab)_2$ immunotoxin (FN18-$F(Ab)_2$-CRM9), or treated with phosphate buffered saline (PBS). The results of the experiment are shown in Figure 2. FN18-CRM9 induced cytokine gene expression for IL2, IL4, IL10, and INFγ, whereas FN18-$F(Ab)_2$-CRM9 selectively induced only IL4 and IL10 expression. In the presence of low doses of DSG (2.5 μg/ml), FN18-CRM9 induction of IL2, 4, 10 and INFγ is reduced. In contrast, low doses of DSG in combination with FN18-$F(Ab)_2$-CRM9, IL4 and IL10 are enhanced, which is consistent with the *in vivo* data in Example 3. Thus, FN18-$F(Ab)_2$-CRM9 in combination with DSG has a unique effect of blocking INFγ expression (consistent with the inhibitory effect of DSG on NF-κB translocation) while enhancing expression of IL4 and IL10. Thus DSG, unlike cyclosporin or FK506, preserves and increases NF-AT transcription factors activation of IL4 and IL10 but selectively inhibits NF-κB dependent cytokine activation pathways.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Although the present invention is described with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the invention except as and to the extent that they are included in the accompanying claims.

REFERENCES

1. Andoins et al. (1996), *Transplantation* 62:1543-1549.

2. Posselt et al.(1990), *Science* 249:1293-1295.

3. Remuzzi et al. (1991), *Lancet* 337:750-752.

4. O'Doherty et al. (1997), *J. of Immunol. Meth.* 207:185-194.

What is claimed is:

1.. A method of inducing immune tolerance to a graft in a recipient, comprising:

(a) administering to the recipient an immunotoxin, thereby reducing the recipient's T-cell population; and

(b) administering to the recipient an agent that inhibits dendritic cell maturation.

2. The method of claim 1, wherein the graft is selected from the group consisting of kidney, liver, heart, pancreas, lung, skin, and isolated cell transplants of pancreatic islets, hepatocytes, stem cell precursors, and differentiated stem cell precursors.

3. The method of claim 1, wherein the agent that inhibits dendritic cell maturation is an inhibitor of nuclear translocation of NfκB.

4. The method of claim 3, wherein the inhibitor of nuclear translocation of NfκB is an analog or derivative of deoxyspergualin.

5. The method of claim 1, wherein the agent that inhibits dendritic cell maturation activates one or more NF-AT dependent Th2 cytokines.

6. The method of claim 1, wherein the agent that inhibits dendritic cell maturation inhibits one or more NfκB dependent Th1 cytokines.

7. The method of claim 1, wherein the inhibitor of dendritic cell maturation is a soluble IL-17 receptor Fc fusion protein.

8. The method of claim 1, wherein the inhibitor of dendritic cell maturation is a glucocorticoid.

9. The method of claim 1, wherein the inhibitor of dendritic cell maturation is a blocker of tumor necrosis factor alpha binding.

10. The method of claim 1, wherein the inhibitor of dendritic cell maturation is a blocker of granulocyte macrophage colony stimulating factor binding.

11. The method of claim 1, wherein the inhibitor of dendritic cell maturation is a blocker of IL-12p70 binding.

12. The method of claim 1, wherein the inhibitor of dendritic cell maturation is a blocker of interleukin 1β binding.

13. The method of claim 1, wherein the inhibitor of dendritic cell maturation is anti-CD154 ligand.

14. The method of claim 1, wherein the agent that inhibits dendritic cell maturation is administered to the recipient at least once.

15. The method of claim 1, wherein the agent that inhibits dendritic cell maturation is administered to the recipient prior to transplantation.

16. The method of claim 1, wherein the immunotoxin is an anti-T cell immunotoxin directed at the CD3 epitope.

17. The method of claim 1, further comprising administering to a transplant donor, prior to harvesting the transplant, an agent that inhibits dendritic cell maturation.

18. A method of screening for an agent that acts synergistically with an immunotoxin in inducing immune tolerance, comprising:

(a) transplanting a donor graft to a recipient;

(b) administering to the recipient an immunotoxin, thereby reducing the recipient's T-cell population;

(c) administering to the recipient the agent to be screened;

(d) obtaining a dendritic cell-containing sample; and

(e) determining a percentage of the dendritic cells in the sample that express or can be induced to express a marker specific for mature dendritic cells, wherein a low percentage shows a synergistic action.

19. A method of screening for an agent that inhibits dendritic cell maturation, comprising:

(a) obtaining a population of immature dendritic cells from a dendritic cell-containing sample of a subject;

(b) culturing the population of cells in the presence of the agent to be screened; and

(c) determining a percentage of dendritic cells that express or can be induced to express a marker specific for mature dendritic cells, wherein a low percentage shows inhibition of dendritic cell maturation.

20. A composition comprising an immunotoxin and an agent that inhibits dendritic cell maturation.

21. The composition of claim 18, wherein the immunotoxin is an anti-T cell immunotoxin directed at the CD3 epitope.

22. The composition of claim 19, wherein the agent that inhibits dendritic cell maturation is an inhibitor of nuclear translocation of NfκB.

23. The composition of claim 19, wherein the agent that inhibits dendritic cell maturation activates one or more NF-AT dependent Th2 cytokines.

24. The composition of claim 19, wherein the agent that inhibits dendritic cell maturation inhibits one or more NfκB dependent Th1 cytokines.

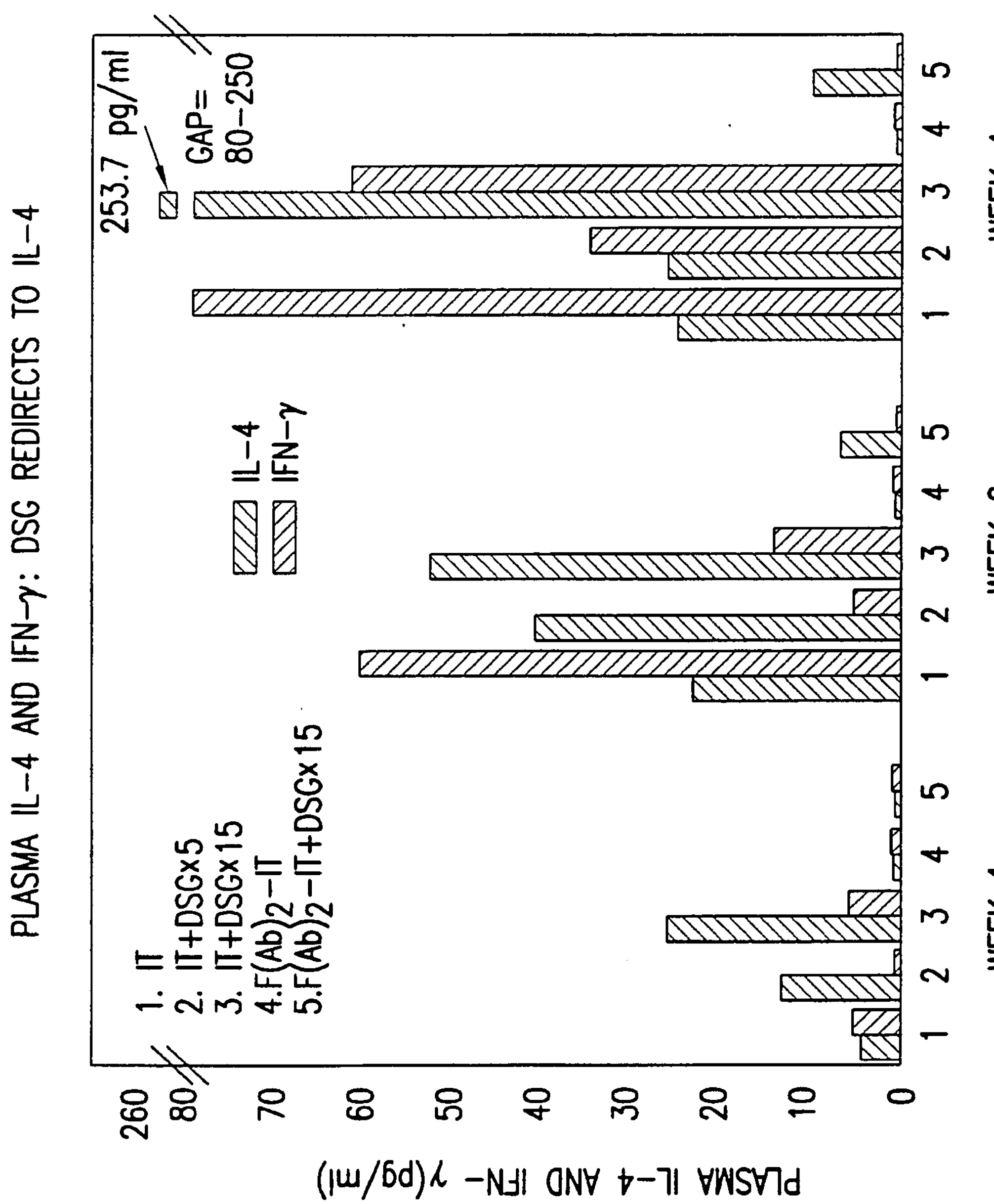

PLASMA IL-4 AND IFN-γ: DSG REDIRECTS TO IL-4
1. IT
2. IT+DSGx5
3. IT+DSGx15
4. F(Ab)2-IT
5. F(Ab)2-IT+DSGx15
IL-4
IFN-γ
253.7 pg/ml
GAP= 80-250
PLASMA IL-4 AND IFN- γ(pg/ml)
260
80
70
60
50
40
30
20
10
0
1 2 3 4 5
WEEK 1
WEEK 2
WEEK 4

FIG.1

2/3

RT–PCR of Cytokine mRNA expression in monkey PBL treated by anti–CD3 immunotoxin

DSG–

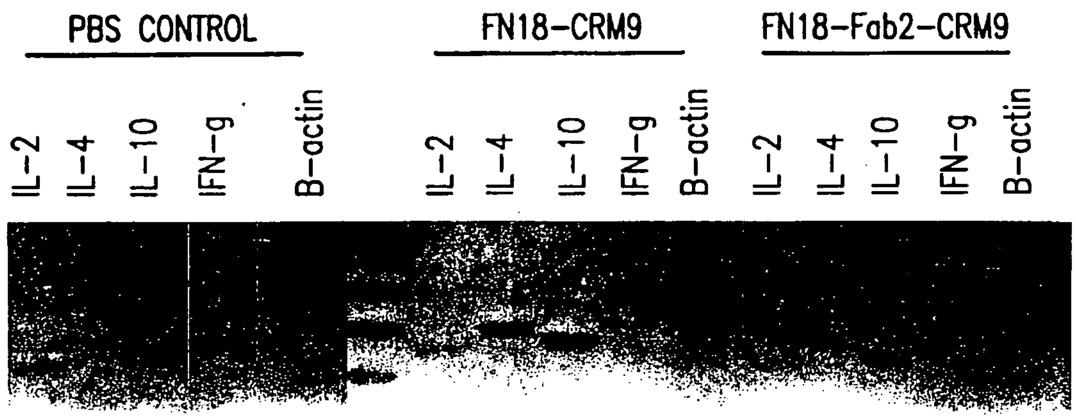


FIG.2A

DSG+

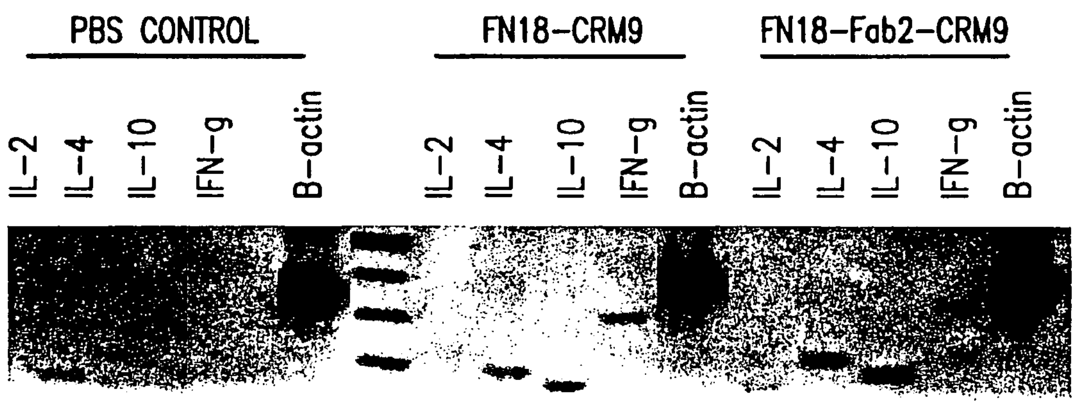


FIG.2B

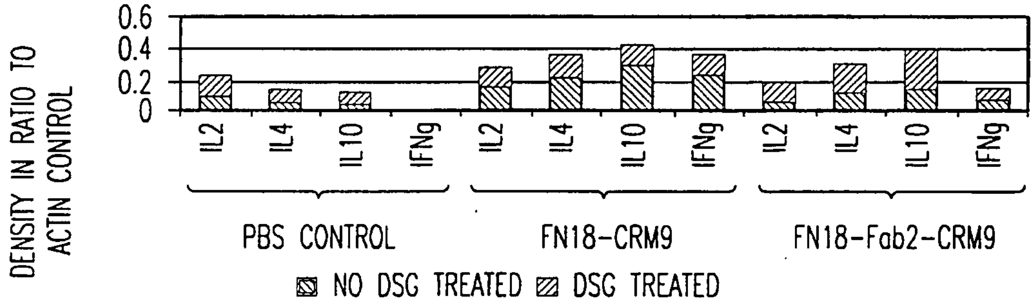

DENSITY IN RATIO TO ACTIN CONTROL
0.6
0.4
0.2
0
IL2
IL4
IL10
IFNg
PBS CONTROL
FN18-CRM9
FN18-Fab2-CRM9
NO DSG TREATED
DSG TREATED

FIG.2C

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/10253

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/155 A61K39/395 A61P37/02 //(A61K39/395,31:155)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, MEDLINE, CHEM ABS Data, WPI Data, PAJ, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 5 736 536 A (CHACE DANA ET AL) 7 April 1998 (1998-04-07) column 5, line 29 - line 40 tables 3,4 ---	18-24 1-17
Y	WO 98 39363 A (HU HUAIZHONG ;MA SHENGLIN (US); US HEALTH (US); KNECHTLE STUART (U) 11 September 1998 (1998-09-11) abstract ---	1-17
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 August 2000	10/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bonzano, C

5

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	ECKHOFF D E (REPRINT) ET AL: "Synergy of 15- deoxyspergualin with antiCD3 immunotoxin in tolerance induction in rhesus monkeys" TRANSPLANTATION, (15 MAY 1999) VOL. 67, NO. 9, PP. 60-60. PUBLISHER: LIPPINCOTT WILLIAMS & WILKINS, 227 EAST WASHINGTON SQ, PHILADELPHIA, PA 19106. ISSN: 0041-1337., XP000933459 UNIV ALABAMA, DEPT SURG, TRANSPLANT CTR, BIRMINGHAM, AL 35294 the whole document ---	1-24
P,X	THOMAS JUDITH M ET AL: "Peritransplant tolerance induction in macaques: Early events reflecting the unique synergy between immunotoxin and deoxyspergualin." TRANSPLANTATION (BALTIMORE), vol. 68, no. 11, 15 December 1999 (1999-12-15), pages 1660-1673, XP000925747 ISSN: 0041-1337 page 1661, column 1, paragraph 2 ---	1-24
X	HAGGERTY H G (REPRINT) ET AL: "Effect of deoxyspergualin or CTLA4Ig on the immunogenicity and pharmacokinetics of the immunotoxin BR96sFv-PE40 in dogs." JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (JAN 1997) VOL. 99, NO. 1, PART 2, SUPP. 'S!, PP. 708-708. PUBLISHER: MOSBY-YEAR BOOK INC, 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, M 63146-3318. ISSN: 0091-6749., XP000925712 BRISTOL MYERS SQUIBB, SYRACUSE, NY;BRISTOL MYERS SQUIBB, NEW BRUNSWICK, NJ the whole document ---	1-24
X	CONTRERAS JUAN L ET AL: "Peritransplant tolerance induction with anti-CD3-immunotoxin: A matter of proinflammatory cytokine control." TRANSPLANTATION (BALTIMORE), vol. 65, no. 9, 15 May 1998 (1998-05-15), pages 1159-1169, XP000925746 ISSN: 0041-1337 page 1159, column 1, paragraph 1 - paragraph 3 page 1160, column 2, paragraph 4 --- -/--	1-24

5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/10253

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	SIEGALL C.B. ET AL: "Prevention of immunotoxin -mediated vascular leak syndrome in rats with retention of antitumor activity." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994) 91/20 (9514-9518). , XP000929449 page 9516, column 1, paragraph 3 -column 2, paragraph 1 page 9518, paragraph 1 - paragraph 3	18-24
A	US 5 801 193 A (OJO-AMAIZE EMMANUEL A ET AL) 1 September 1998 (1998-09-01) column 7, line 10 - line 55	
A	US 5 747 474 A (OJO-AMAIZE EMMANUEL A ET AL) 5 May 1998 (1998-05-05) column 5, line 43 - line 65	

5

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/10253

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5736536 A	07-04-1998	US 5688781 A	18-11-1997
		CA 2156579 A	20-02-1996
		EP 0701817 A	20-03-1996
		JP 8169821 A	2-07-1996
WO 9839363 A	11-09-1998	AU 6542698 A	22-09-1998
		EP 1015496 A	05-07-2000
US 5801193 A	01-09-1998	AU 7142998 A	11-11-1998
		EP 0986381 A	22-03-2000
		WO 9846222 A	22-10-1998
US 5747474 A	05-05-1998	AU 3814497 A	20-02-1998
		EP 0956025 A	17-11-1999
		WO 9804271 A	05-02-1998
		US 5968912 A	19-10-1999

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-3,5-24 relate to a compound defined by reference to a desirable characteristic or property, namely the activity as inhibitor of dendritic cell maturation.

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds mentioned in claim 4 and the deoxyspergualin-derivative specifically disclosed in the description at page 3, with due regard to the general idea underlying the present invention.

Claims searched completely: 4.
Claims searched incompletely: 1-3, 5-24.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.